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Profiling of Microrna Expression within the Cells of Peripheral Blood Mononuclear after an Infection with Serotype-2 of Dengue Virus: Preliminary Study

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The role of micro Ribo Nucleic Acids (miRNA), a small-non coding RNA has been associated with immune regulation in various viral infection including dengue infection. The microRNA will bind a specific protein target in order to encourage an explosive expression of various cytokines, known as cytokines storm in Dengue infection. The objective of this study aimed to determine and evaluate the microRNAs profile expression within peripheral blood mononuclear cells having been infected with one of the dengue virus serotype. To obtained the PBMCs from a healthy donor, Ficoll density gradient centrifugation was used to isolate the PBMCs and then followed infecting it with a DENV-2 clinical isolate. Prior to PBMCs isolation, the virus has been propagated and having titration to get an optimal virus titer. We conducted the infection at the multiplication of infections 4 PFU/10^6 cells. MiRCURY LNA™Exiqon was utilized on purpose to extract the RNA. Quantitative Real-Time PCR was applied in order for the miRNAs relative expression to be measured. The preliminary result reveals that miR-150, miR-146a, hsa-let-7e expression were increased 1.74 folds, 2 folds, and 1.49 folds respectively at 12 hours post-infection on PBMCs upon DENV-2 infection. The expression of microRNAs was discovered to be higher in PBMCs at the time of infection with DENV-2. The miRNAs expression in the uninfected PMBCs was lower than that of the miRNA. This high expression of miRNAs in dengue infection may proceed to dengue infection pathogenesis.

Keyword: miRNA; Expression; Dengue; Infection.

The prevalence of DENV infection in Asia-Pacific region has been reported as hyper endemic in which all the serotypes of the virus were discovered circulating within the countries.[1-4] The most dengue presentations mostly mild form, but actually it were identified to be widely varied from asymptomatic, mild dengue fever (DF), and dengue hemorrhagic fever (DHF), including the dengue shock syndrome which is life-threatening (DSS).[5] The mild dengue manifestations (DF) are fever accompanied by a severe headache, retro-orbital pain, arthralgia, myalgia, rash and minor hemorrhage such as petechiae, epistaxis or gum bleeding. In contrast with more severe dengue, dengue hemorrhagic fever (DHF) is characterized by all symptom of DF and occurrence of plasma leakage, such as hemoconcentration, ascites, or pleural effusion.[5] Severe plasma leakages lead the patients fall to DSS with relatively high mortality rate particularly in the population such as younger, elderly, pregnant, or the patients with pre-existing illnesses. Despite many studies

having been conducted, dengue pathogenesis is not fully understood. In addition, several studies have explored the factors to predict the disease progression between DF to DHF or DSS.[6–9] Studies in Southeast Asian countries found the issues related to the dengue severity are immune status, genetics of the population, viremia titers, serotypes divergences, and the abundant release of cytokines.[8,10,11] The non-neutralizing antibody of the previous dengue virus infection, dengue serotype-2 found to be the critical role to get severe DENV infection.[12,16] The role of cytokines in dengue pathogenesis has been related to the process of leakage within the endothelial cell.[13–16] The elevated levels of IL-6, IL-10, IFN-ã, MIF, and CCL-4 might be used as a potential predictors to get severe dengue.[17–21] The study of cytokines gene expression within human peripheral blood monocyte-derived macrophages having been infected with dengue virus serotype-2 which found IL-8, IL-1b, osteopontin, GRO-a, -b and -g, I-309 genes expression, was significantly larger compared to that of the uninfected.[22]

The expression of cytokines encoding gene may be controlled by several number of endogenous or exogenous factors. A small, short, non-coding RNA, contains an 18-22 nucleotide named microRNA which has been found to be functional primarily by attaching on the 3'UTR un-translated area of mRNA target, resulting in impediment of translation or mRNA degradation.[23]The miRNA has been predicted as a regulator of cytokine gene expression by binding a specific protein, resulting in uncontrolled expression of the cytokine gene. The Role of miRNA in the production of cytokines regulation, cytokine which signals and facilitates the stability of Th1/Th2 polarization, has been studied elsewhere.[23,24]The protein miRNAs such as miR-146a, miR-30e*, miR150, miR-548g-3p as well as the others are induced by DENV and associated with DENV replication or cytokines induction.[25–29]The current study reports the miR-150 expression, has-let-7e and miR-30e* in infected PBMCs versus non-infected with local clinical isolate (Semarang DENV-2 isolate).

MATERIALS AND METHODS

This research was assessed as well as accepted by Institutional Review Board of the Faculty of Medicine, Udayana University (Approval No:485/UN.14.2/KEP/2017). The study was an experimental study in regards to peripheral blood mononuclear cells having been infected with dengue virus serotype-2. The DENV-2 was isolated from the local sample (Semarang Indonesia isolate). Sub-cultured propagating the virus was conducted in C6/36 mosquito cells. In addition, the virus titer was verified through plaque assay as depicted in the prior report.[31] Prior to the process of obtaining blood samples being performed, a healthy donor was given written consent. By utilizing Ficoll-Hypaque density gradient centrifugation, PBMCs were placed in isolation. All the PBMCs are re-suspended through identical medium with 10% inactivated fetal calf serum after having been sterilized by using RPMI-1640 medium, Culturing and incubating the PBMCs at 37°C in 5% CO_2 for 6-12 hours was the last procedure conducted in the process. Infecting the PBMCs with DENV-2 within medium with the infection multiplicity (MOI) was then performed following the procedure mentioned above. The cells were placed in the incubation at 37°C and sampled at 6, 12 and 24 hours post-infection. Virus growth characteristic in the cell line was determined with a viral kinetic assay in duplications. MiRCURY LNA™Exiqon (Sweden) RNA isolation kit was used in a single step as described by the kit manufacturer's instructions in order for the RNA to be able to be totally extracted. The RNA quality and quant were evaluated with Qubit 3 fluorophotometer. The internal reference genes were non-coding nuclear RNA (snRNA U6) for miRNA which are small in size. In order to be able to analyze the relative miRNA expression, Quantitative real-time polymerase chain reaction (qRT-PCR) (Biorad, USA) was applied in the process. Total RNA of the donor was used for detection of miRNAs has-let-7e, miR-30e*, miR-150, miR-146a and has-miR-4290.

RESULTS

The supernatants were obtained at 6, 12 and 24 hours post DENV-2 infection. At the 12th observation of the observation time, the expression of miR-150 was found up-regulated 1.78 folds which are higher than the uninfected sample. The miRNA hsa-let-7e was expressed 1.49 folds at 12th h post infection in infected samples compared

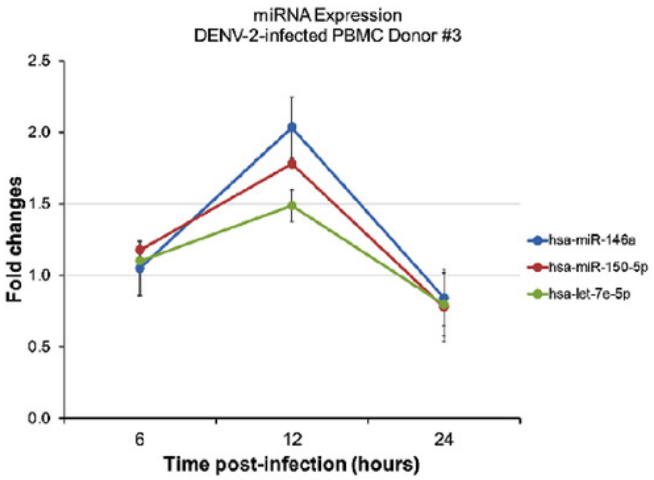

Fig. 1. Expression of miRNAs in PBMCs infected with DENV-2

with that of the uninfected sample. The expression of miR-146a was discovered as high as 2 folds in 12th h post infection compared with the uninfected sample. On the other hand, the miRNA, miR-30e* and miR-4290 were not expressed as high as the other miRNA (data not shown) (Figure 1).The target gene of the miRNA such as suppressor of cytokine signaling (SOCS-1 or SOCS-3) was also evaluated in the current study. In addition, it was detected that the immunological responses of the PBMCs were stimulated by DENV-2 infection through the qRT-PCR expression profiling of genes encoding cytokines and chemokine namely IL-6, IL-8, IP-10 and MIP-1â (data not shown).

DISCUSSION

Vascular leakage is a hallmark of severe dengue manifestation which is a leading factor in morbidity and mortality in dengue shock syndrome.[15,30] Elevation of several cytokines in dengue infection has been widely correlated with the vascular leakage in which the fluid accumulation on the third space such as pleural effusion, ascites or even shock, may present as the manifestation of plasma leakage.[33,34] Control of cytokine overproduction by the protein such as SOCS may be lost due to the binding of this protein by particular miRNA. Up- regulation of miR-150 has been reported to correlate with low expression of SOCS-1 in the leucocyte patient with DHF.[26] In our study, although the expression miR-150 was not as high as with another study which was expressed 7.16 folds in the serum samples of DHF than DF (p=0.008).[26] This discovery seems to support the involvement of miR-150 within the pathogenesis of DENV infection. This difference across all types of miR-150 expression may possibly be due to the difference in terms of microRNA time points sampling procedure, different DENV infection methods conducted in our research, which is lower compared to the previous study.[26] High expression of miR-146a, miR-30e*, miR-150, miR548g-3p in DENV infection which suggests the role important of miRNA in the DENV pathogenesis has been reported.[28,35,36]

In contrast, the low expression of miR-4290 and miR-30e* in PBMCs (data not shown) is contrasted with the previous study.[27,35] The PBMCs are unstable cells (mortal cell) which even reflect closer in-vivo, may explain this discrepancy with high expression of miR-30e* which was conducted in the human monocyte U937 cell line.[35] Meanwhile, the expression of miR-146a is supported by another study.[36] In this study the highest expression of miR-146a was only 2-fold at the 12 h post infection in PMBCs, meanwhile another study found the expression as high as

9-fold, 10-fold and 10-fold at 12 h, 24 h and 48 h p.i in human monocyte. We do not able to isolate the monocyte from the PBMCs, which may explained why the expression of the miR-146a is not as high as another study in human monocyte. Since the report is a preliminary report, further data need to be brought into submission and reported in order to support the miRNA role in DENV infection. To summarize all the details, our research emphasizes the respective role of miRNA in DENV in infected PBMCs which support other discoveries of miRNA expression in order to find out the new DENV infection pathogenesis.

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